



SMA-1 spectrin has essential roles in epithelial cell sheet morphogenesis in *C. elegans*

Vida Praitis^{a,*}, Emily Ciccone^a, Judith Austin^{b,1}

^aBiology Department, Grinnell College, Grinnell, IA 50112, USA

^bDepartment of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637, USA

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Abstract

During *Caenorhabditis elegans* development, the embryo acquires its vermiform shape due to changes in the shape of epithelial cells, a process that requires an apically localized actin cytoskeleton. We show that SMA-1, an ortholog of β_H -spectrin required for normal morphogenesis, localizes to the apical membrane of epithelial cells when these cells are rapidly elongating. In *spc-1* α -spectrin mutants, SMA-1 localizes to the apical membrane but its organization is altered, consistent with the hypothesis these proteins act together to form an apically localized spectrin-based membrane skeleton (SBMS). SMA-1 is required to maintain the association between actin and the apical membrane; *sma-1* mutant embryos fail to elongate because actin, which provides the driving force for cell shape change, dissociates from the apical membrane skeleton during morphogenesis. Analysis of *sma-1* expression constructs and mutant strains indicates SMA-1 maintains the association between actin and the apical membrane via interactions at its N-terminus and this activity is independent of α -spectrin. SMA-1 also preserves dynamic changes in the organization of the apical membrane skeleton. Taken together, our results show the SMA-1 SBMS plays a dynamic role in converting changes in actin organization into changes in epithelial cell shape during *C. elegans* embryogenesis.

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Introduction

Morphogenesis, the process by which an organism or tissue acquires its mature shape, is fundamental to embryonic development. In *Caenorhabditis elegans*, changes in epithelial cell shape are critical for establishment of the mature larval shape. Morphogenesis of the hypodermis, the external epithelium, occurs in three sequential steps involving cell intercalation, cell migration, and cell shape change. The hypodermal cells of *C. elegans* are born on the dorsal

surface of the embryo and rearrange by intercalation (Podbilewicz and White, 1994; Priess and Hirsh, 1986; Sulston et al., 1983; Williams-Masson et al., 1998). The hypodermal sheet then migrates ventrally, enclosing the embryo in a single layer of cells (Raich et al., 1999; Williams-Masson et al., 1997). Once enclosure is complete, the relative positions of the hypodermal cells do not change. In the final stage of morphogenesis, the hypodermal cells elongate, changing in shape from columnar to rectangular along the anterior–posterior axis of the embryo, increasing the length of the *C. elegans* embryo fourfold (Priess and Hirsh, 1986).

An apically localized cytoskeleton is essential for hypodermal cell shape change during *C. elegans* morphogenesis. After enclosure, cortical arrays of actin in the hypodermis dramatically reorganize to form parallel apically localized bundles that circumscribe the embryo. As the embryo elongates, the number of actin bundles remains constant, but the distance separating them increases (Costa

Abbreviations: CH, calponin homology; PH, pleckstrin homology; SH3, Sarc homology 3; SBMS, spectrin-based membrane skeleton; SR, spectrin repeat; GFP, green fluorescent protein; CFs, circumferential actin fibers; AJ, adhesion junctions.

* Corresponding author.

E-mail address: praitis@grinnell.edu (V. Praitis).

¹ Current Address: Judith Austin, Virginia Commonwealth University, Microbiology and Immunology, BOX 980678, 1101 E. Marshall Street 5TH, Richmond, VA 23284, USA.

et al., 1997; Priess and Hirsh, 1986). Disruption of the actin cytoskeleton with cytocholasin D prevents embryonic elongation (Priess and Hirsh, 1986). Actin interacts with two non-muscle myosins NMY-1 and NMY-2, producing the contractile force that drives cell shape change. Several proteins that regulate this contractile activity have been identified in *C. elegans*, including MLC-4/myosin light chain kinase, MEL-11/smooth muscle myosin phosphatase, and LET-502/rho binding kinase. Disruption of genes encoding these proteins perturbs embryonic elongation, although organization of the actin filaments is unperturbed (Piekny et al., 2000; Piekny et al., 2003; Shelton et al., 1999; Wissmann et al., 1999).

Proteins that interact with actin fibers at apical hypodermal cell boundaries during morphogenesis have been identified and are components of the *C. elegans* apical adhesion junctions (AJ). In *C. elegans*, two subdomains define the AJ. The more apical adhesion complex consists of HMP-1/ α -catenin, HMP-2/ β -catenin, HMR-1/cadherin, VAB-9/BCMP1/claudin, APR-1/APC-related, and JAC-1/p120 catenin (Costa et al., 1998; Hoier et al., 2000; Pettitt et al., 2003; Raich et al., 1999; Simske et al., 2003). A second more basal adhesion complex is formed by the interactions of DLG-1/*discs large* and AJM-1, a novel protein (Bossinger et al., 2001; Firestein and Rongo, 2001; Koppen et al., 2001; Legouis et al., 2000). Establishment and maintenance of the AJ and, in the gut, the integrity of the apical membrane, requires LET-413/*Scribble* (Legouis et al., 2000; Koppen et al., 2001; Bossinger et al., 2001; McMahon et al., 2001; Bossinger et al., 2004; Segbert et al., 2004). Mutations in AJ genes cause embryonic arrest, with defects in cell migration, ventral closure, and cell elongation (Bossinger et al., 2001; Costa et al., 1998; Firestein and Rongo, 2001; Hoier et al., 2000; Koppen et al., 2001). One critical role for the adhesion junction during cell elongation is to link the actin fibers to the apical cell boundaries, as these fibers dissociate from the adhesion junction in *hmp-1*, *vab-9*, and *dlg-1(RNAi)* embryos (Costa et al., 1998; Firestein and Rongo, 2001; Pettitt et al., 2003; Simske et al., 2003). However, proteins responsible for organizing and tethering actin filaments along the surface of the apical membrane have not been characterized.

One candidate for a protein that links actin to the apical membrane of epithelial cells during morphogenesis is SMA-1 spectrin, an ortholog of *Drosophila* β_H -spectrin. β -spectrins, first identified in erythrocytes, contain a number of protein domains, including the Calponin Homology (CH), Sarc homology 3 (SH3), spectrin repeat (SR), and pleckstrin homology (PH) domains thought to be important for protein interactions (reviewed in Bennett and Baines, 2001; De Matteis and Morrow, 2000). β -spectrins interact with α -spectrins to form heterotetramers that cross-link actin at cell membranes. These spectrin-based membrane skeletons (SBMS) stabilize cell membranes against mechanical stress (Bennett and Baines, 2001). Unlike the conventional β -spectrin UNC-70, which acts in synapse function, muscle

structure, and in axon path finding (Hammarlund et al., 2000; Moorthy et al., 2000), SMA-1 plays roles in epithelial cell shape change. Mutations in *sma-1* result in global cell shape defects in the hypodermis, gut, pharynx, and excretory canal cell (Buechner et al., 1999; McKeown et al., 1998) similar to but less severe than embryos treated with cytocholasin D (Priess and Hirsh, 1986). A null mutation in *spc-1*, the α -spectrin gene of *C. elegans*, produces a phenotype that resembles β - and β_H -spectrin double mutants, indicating the activity of each of these proteins depends at least in part on the formation of spectrin heterotetramers (McKeown et al., 1998; Moorthy et al., 2000; Norman and Moerman, 2002). In *Drosophila*, mutations in the β_H -spectrin gene cause cell shape change and cell adhesion defects for a number of tissues (Thomas et al., 1998; Zarnescu and Thomas, 1999). A recently cloned homolog of β_H -spectrin (named β_V spectrin; Stabach and Morrow, 2000) may play a similar role in humans. In *C. elegans*, the actin cytoskeleton in hypodermal cells is disrupted in *spc-1* and *sma-1* null mutants (Norman and Moerman, 2002), indicating the α/β_H -spectrin heterotetramer mediates changes in cell shape through its interactions with actin. However, the mechanisms by which SMA-1 spectrin affects cell shape change and the precise interactions between SMA-1 spectrin and actin have not yet been determined.

In an effort to understand the mechanisms that regulate epithelial cell shape change in *C. elegans*, we characterized SMA-1 spectrin and several classes of *sma-1* mutants. We have determined SMA-1 spectrin plays several distinct roles in epithelial morphogenesis. SMA-1 localizes to the apical membrane of elongating epithelial cells, where it is an essential part of the SBMS that stabilizes changes in the membrane skeleton. SMA-1 also interacts with the circumferential actin fibers (CFs) present during morphogenesis and plays a critical role in maintaining the association of these fibers with the apical plasma membrane during cell elongation. The ability of SMA-1 to maintain the interaction between actin and the apical membrane resides in the amino-terminus of SMA-1 protein and this activity is independent of α -spectrin. We propose the SMA-1 SBMS is dynamic and flexible and reorganization of this network is essential for cell shape change during *C. elegans* morphogenesis.

Materials and methods

C. elegans strains, growth conditions, and length measurements

Strains were grown at 20°C on standard nematode growth media (Brenner, 1974) or on Opti-gro plates supplemented with Nystatin (Praitis et al., 2001). We used wild type *C. elegans* var. Bristol strain N2. All *sma-1* strains used in this study were isolated in F1 clonal or non-complementation screens, using EMS or UV/psoralen mutagens (McKeown et al., 1998). *sma-1(e2616)* and

sma-1(ju7) were kindly provided by J. Hodgkin and A. Chisolm, respectively. All *sma-1* strains are viable and fertile (McKeown et al., 1998). We measured the larval length of *sma-1* mutants within 2 h of hatching (McKeown et al., 1998). Each allele was separated into one of three categories: Weak alleles were >70% of wild type length, intermediate alleles were 60 to 69% of wild type length, and strong alleles, including the *sma-1(ru18)* null allele, were <60% of wild type length. Other strains used in this study: DM3416 mnDp33/+IV; *spc-1(ra409)*X (kindly provided by D. Moerman).

Determining sites of *sma-1* mutations

We used the PCR enzyme Expand (Roche, Indianapolis, IN) to amplify three overlapping fragments of *sma-1* genomic sequence from each *sma-1* strain. The *sma-1* gene was sequenced 5' to 3' until completion, or a premature stop codon was confirmed. To rule out a PCR error, each nucleotide change was confirmed by sequencing a second independently produced PCR fragment. Sequencing was performed at the U. of Chicago Cancer Research Center DNA Sequencing Facility. Eight EMS-induced alleles had a single nucleotide change, indicated in parentheses with the position relative to the ATG start listed after each allele: *ru1* (G/A) 2848, *mu82* (G/A) 3266, *ru3* (C/T) 5793, *e2616* (C/T) 5874, *e30* (G/A) 7002, *mu81* (G/A) 9597, *mu83* (G/A) 12273, and *ru7* (A/T) 1709. The UV/psoralen-induced *ju7* allele had a deletion at 2322–2340 and novel DNA sequence which, if translated, would generated an 18 amino acid insert before going out of frame: MFFTIQICSSQSRL-KEKR.STOP (Table 1).

Creation of GFP fusion strains using microparticle bombardment

We used microparticle bombardment to generate lines carrying transgenic constructs (Praitis et al., 2001). All plasmids described contain sequence necessary to rescue the

Unc-119 phenotype and strains are *unc-119(ed3)*. AZ249, an obligate heterozygote, carries integrated plasmid pAZ146, which contains the *pie-1* promoter linked to an actin and green fluorescent protein (GFP) fusion (kindly provided by G. Seydoux; Chalfie et al., 1994). During morphogenesis, this strain expresses actin::GFP in the hypodermis but not in muscle cells. The AZ220 SR4::GFP line carries integrated plasmid pAZ110, which has 3.3 kb of *sma-1* 5' regulatory region and UTR, 3.6 kb of *sma-1* coding sequence, fused in frame to GFP, and the *unc-54* 3' UTR. The sequence encodes the SMA-1 N-terminus including the first 16 amino acids of spectrin repeat (SR) 5. AZ258 was made by crossing *sma-1(ru18)* animals with AZ220, and obtaining F2 *sma-1* mutants that expressed SR4::GFP. We crossed DM3416 with AZ220 to obtain F2 *spc-1* mutants that expressed SR4::GFP.

Generation of anti-SMA-1 antisera and immunoblotting

We made bacterial fusion proteins containing partial SMA-1 sequence, fused to 6 histidines. The 2.3-kb *SacI* to *PstI* fragment of *sma-1*, from SR27 to the end of the protein, was used to make the PH antigen, and a 0.7-kb *BamHI* to *HindIII* fragment of *sma-1*, from SR5 to SR6, was used to make the SH3 antigen. The bacterial fusion proteins were produced to near homogeneity and isolated on Ni-NTA columns (Quiagen, Valencia, CA) followed by dialysis. To produce antisera, each antigen was injected into two rabbits (Covance, Denver, PA). In N2 strains, all 4 antisera had similar immunofluorescence staining patterns in situ and identified a band of ~470 kDa (the predicted size of SMA-1) on protein immunoblots (Fig. 1). We also observed additional stained bands on immunoblots, different for each of the 4 antisera (data not shown). For immunoblots examining expression of SMA-1 protein in *sma-1* alleles, we used affinity-purified anti-SH3 or anti-PH domain antisera. To compare antisera signal in N2 wild type and *sma-1* mutant strains, we loaded protein from an equal numbers of embryos in each lane and confirmed that equal

Table 1
Characterization of *sma-1* mutants

| Class | Allele | Amino acid change | Mutation location | Actin organization ^a | Larval length phenotype ^b |
|-------|--------------|-------------------|-------------------|---------------------------------|--------------------------------------|
| I | <i>ru18</i> | Deletion/stop | CH1 | Disorganized | Strong |
| II | <i>ju7</i> | Deletion/stop | SR1 | Organized | Weak |
| | <i>ru1</i> | W558stop | SR2 | Organized | n.d. |
| | <i>mu82</i> | W647stop | SR4 | Organized | Weak |
| III | <i>ru3</i> | Q1474stop | SR11 | Disorganized | Intermediate |
| | <i>e2616</i> | Q1501stop | SR11 | Disorganized | n.d. |
| | <i>e30</i> | W1860stop | SR14 | Mixed ^c | Intermediate |
| | <i>mu81</i> | W2624stop | SR22 | Disorganized | Intermediate |
| | <i>mu83</i> | W3459stop | SR30 | Mixed ^c | Weak |
| IV | <i>ru7</i> | I558F | CH2 | Novel | Weak |

n.d., Not determined.

^a See also Figs. 3 and 7.

^b See Materials and methods for phenotype classification.

^c Strains classified as mixed had some embryos with organized fibers and others with disorganized fibers.

amounts of total protein were present on the blots by Ponceau S staining. In *sma-1(ru18)* null embryos, no SMA-1-specific staining was observed in situ or on immunoblots (Figs. 1M, N), indicating the antisera are specific for SMA-1 protein.

Immunofluorescence and live animal microscopy

To stain embryos, we used affinity-purified rabbit anti-*sma-1* PH antisera 38-F at a 1:333 dilution and MH27, which recognizes AJM-1 at a 1:250 dilution, followed by Alexa-goat anti-rabbit or anti-mouse antisera at 1:250 dilutions (Molecular Probes, Eugene, OR; Koppen et al., 2001; McKeown et al., 1998; Priess and Hirsh, 1986). To

visualize filamentous actin, embryos were stained with Alexa-phalloidin (Molecular Probes; Costa et al., 1997). The preparation method used for phalloidin staining was not compatible with the anti-SMA-1 antisera. To examine the association of actin with the plasma membrane, we grew the strain AZ249 on OP50 bacteria or on bacteria transformed with *sma-1* (RNAi) construct pAZ171, which contains 2.1 kb of *sma-1* sequence, from nucleotide 3760 to 5873 (Kamath et al., 2000). For all RNAi experiments, we selected embryos from plates on which all larvae had the *Sma* phenotype. Eggs were collected into a 6- μ l drop of embryo media containing Schneider's salt, fetal bovine serum, egg salts buffer (Mohler and White, 1998), 2,3-butanedione monoxime to paralyze the animals (Miriam Goodman and Marty Chalfie, personal communication), and FM4-64 (Molecular Probes) to visualize the plasma membrane. The embryo eggshells were broken by adding a cover slip. Animals were photographed within 30 min of treatment.

To determine the localization pattern of *sma-1::GFP* fusion protein in wild type, *sma-1(ru18)* and *spc-1(ra409)* strains, 21 embryos from each strain were placed on agarose pads in egg buffer (Edgar, 1995) and scored for GFP signal and localization. For *spc-1* mutants, 21/21 embryos had apically localized SR4::GFP. Of these, 7 failed to elongate properly and hatched with the *Sma* phenotype, indicating they were homozygous for *spc-1*. It was not possible to both

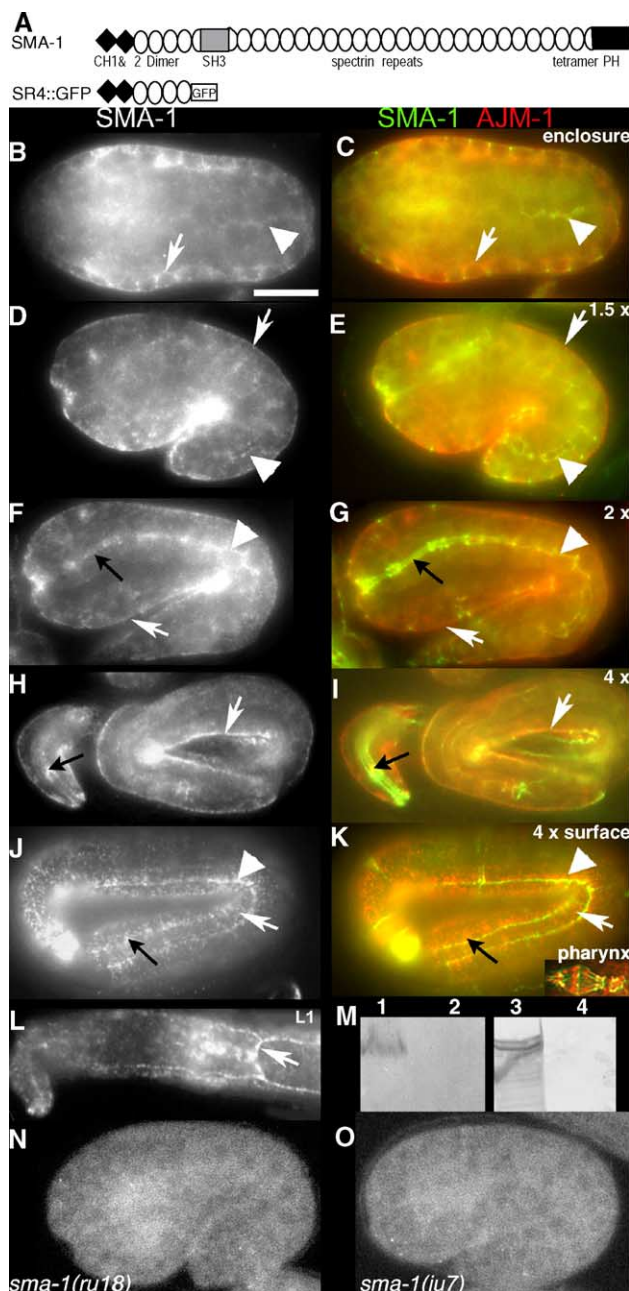


Fig. 1. SMA-1 localizes to the apical membrane in the hypodermis, gut, pharynx, and excretory canal cell during cell elongation. (A) A schematic of SMA-1 spectrin, which contains CH1 and CH2, followed by SR1–4, an SH3 in SR5, SR6–28, a partial SR likely important for tetramerization (Bennett and Baines, 2001), and a PH domain. Shown below is the SMA-1 SR4::GFP construct, which includes the N-terminus of SMA-1 including a few amino acids in SR5 fused to GFP. To visualize SMA-1 localization, wild type (B–L) animals were co-stained with anti-*sma-1* PH antisera and MH27, which recognizes AJM-1 at apical AJs (Koppen et al., 2001; Materials and Methods). Left panel shows SMA-1, right panels show SMA-1 in red, AJM-1 in green. In all photos, anterior is to the left. (B, C) A coronal section of pre-enclosure embryo shows localization of SMA-1 in the gut (arrowhead) and to all membranes of the hypodermis (white arrow). (D, E) Lateral cross-section of 1.5-fold embryo. SMA-1 is localized to the apical membranes of hypodermal cells (white arrow) and gut (arrowhead). (F, G) Cross-section of ~2-fold stage embryo. SMA-1 localizes to apical cell membranes of hypodermal (white arrow), gut (arrowhead), and pharynx (black arrow) cells, but does not co-localize significantly with AJM-1 (green). (H, I) Cross-section of ~3-fold stage embryo, SMA-1 is localized to the apical cell membranes of hypodermal (white arrow) and pharynx (black arrow) cells. (J, K) Lateral surface view of the same embryo as in H, SMA-1 (red) is expressed in both seam (white arrow) and D/V hypodermis (black arrow), with enrichment at boundaries between these cells (arrowhead) that is distinct from AJM-1, in green. Inset of pharyngeal bulb, showing SMA-1 (red) apical to AJM-1 (green). (L) Ventral cross-section of L1 larva, SMA-1 is apically localized in the excretory canal cell (H-shaped cell, white arrow). (M) Immunoblots stained with anti-SMA-1 PH antisera (lanes 1 and 2) or anti-SMA-1 SH3 antisera (lanes 3 and 4), showing an ~470-kDa protein in extracts from N2 wild type (lanes 1 and 3) but not *sma-1(ru18)* (lane 2) or *sma-1(ju7)* (lane 4) embryos. *sma-1(ru18)* (N) or *sma-1(ju7)* (O) embryos, stained with anti-SMA-1 PH antisera, show no localized expression. Scale bar in panel B, 10 μ m.

photograph and genotype *spc-1* animals carrying the SR4::GFP construct.

For fluorescence microscopy, we used a Zeiss Axioplan or an Olympus BX51. For confocal imaging, we used a Zeiss LSM 510 interfaced to an Axiovert inverted microscope containing a photomultiplier system with three-color lasers (U. of Chicago Confocal Digital Imaging Facility). For the z-stack series, aperture settings and z-step size were identical for all the images shown (Fig. 4). Images were manipulated using Adobe Photoshop 7.0.

Electron microscopy

sma-1 larvae were prepared using standard methods (Hall, 1995). We used a JEOL 840a Scanning Electron Microscope operated at 10 kV (Cancer Research Center Electron Microscope Core Facility, U. of Chicago) to visualize animals.

Results

SMA-1 spectrin is apically localized in the hypodermis, gut, and pharynx during embryonic morphogenesis

To examine the role of SMA-1/ β_H -spectrin in *C. elegans* embryonic morphogenesis, we determined the expression pattern of SMA-1 protein by using antisera we generated against the SMA-1 PH domains (Fig. 1, Materials and methods), which recognizes an ~470-kDa protein from wild type embryos (Fig. 1M). We found SMA-1 spectrin first appears in hypodermal cell precursors located on the dorsal surface of the embryo prior to enclosure (data not shown). SMA-1 is present in the hypodermal cells as they enclose the embryo, and continues to be expressed in the dorsal, lateral, and ventral hypodermal cells throughout embryogenesis (Figs. 1B–K). SMA-1 is also expressed in gut and pharynx cells prior to embryonic enclosure, and throughout embryogenesis (Figs. 1B–K). In *sma-1(ru18)* null mutant embryos, both immunoblot and in situ immunofluorescence staining show no signal (Figs. 1M, N), demonstrating the antiserum is specific for SMA-1 protein.

The protein localization patterns we observe using SMA-1 antisera are consistent with mRNA in situ expression data (McKeown et al., 1998), except in the excretory canal cell, where *sma-1* mRNA is present during embryogenesis (McKeown et al., 1998), but SMA-1 protein expression is delayed until the L1 stage (Fig. 1L). The timing of SMA-1 protein expression precisely correlates with the period when the excretory cell begins to rapidly elongate, during the first larval stage of development. That SMA-1 protein is expressed in the excretory canal cell, hypodermis, gut, and pharynx during the period of rapid cell elongation implies a fundamental role for SMA-1 in cell elongation.

The intracellular location of SMA-1 spectrin is developmentally regulated. During early morphogenesis, as the

hypodermal cells migrate to enclose the embryo, SMA-1 is present at all epithelial cell boundaries, with some protein in the cytoplasm (Figs. 1B, C). Once enclosure is complete, SMA-1 protein localizes to the apical membrane of hypodermal, pharyngeal, and gut cells where it remains during embryonic elongation (Figs. 1D–K). SMA-1 is found in a speckled pattern at the apical surface of the both dorsal/ventral and seam hypodermis, with increased intensity near cell boundaries (Fig. 1K). The reorganization of SMA-1 in the hypodermis is concurrent with a change in the organization of actin, from cortical arrays to parallel bundles associated with the apical membrane (Priess and Hirsh, 1986).

One candidate protein for targeting SMA-1 to the apical membrane is α -spectrin. Based on homology to other systems, the similar mutant phenotypes, and co-localization of these proteins, SMA-1 likely forms a network with α -spectrin at the apical membrane (Bennett and Baines, 2001; De Mattei and Morrow, 2000; McKeown et al., 1998; Norman and Moerman, 2002). Work in *C. elegans* has shown SPC-1 α -spectrin localizes to the apical membrane in *sma-1(ru18)* null mutants (Norman and Moerman, 2002) and *Drosophila* cells lacking α -spectrin have little detectable apical β_H -spectrin (Dubreuil et al., 1998; Lee et al., 1997). To determine if the localization of SMA-1 at the apical membrane requires α -spectrin, we stained the DM3416 embryos with antisera to SMA-1. The DM3416 strain is homozygous for *spc-1(ra409)*, which contains a premature termination sequence in the *spc-1* C-terminus, but carries a balancer containing wild type *spc-1*. Embryos that have lost the balancer are *Sma* and die as early L1 larva (Norman and Moerman, 2002). In early morphogenesis, there is no distinct morphological change in the embryo that allowed us to distinguish *Spc-1* embryos from siblings carrying the balancer. In 1.5-fold DM3416 embryos, 11/11 had apically localized SMA-1, although it appeared more punctate than in the wild type in 5 of these embryos (data not shown). *Spc-1* embryos in late morphogenesis can be recognized because they do not elongate past 2-fold, but have mature AJM-1 organization in the pharynx. 18/20 of these *Spc-1* embryos had apically localized SMA-1, but the pattern was weaker and more punctate than in the wild type (Fig. 2). These data show *spc-1* is not absolutely required for the apical localization of SMA-1 but is required for its normal organization at the apical membrane.

SMA-1 maintains apical localization of actin during cell elongation

In all *sma-1* mutant embryos examined, actin organization appears normal during enclosure and the actin bundles reorganize normally at the beginning of cell elongation (data not shown). However, during cell elongation, actin fibers become disorganized in *sma-1(ru18)* null embryos (Fig. 3; Norman and Moerman, 2002).

To better understand why actin fibers are disorganized, we compared phalloidin localization in *sma-1(ru18)* null

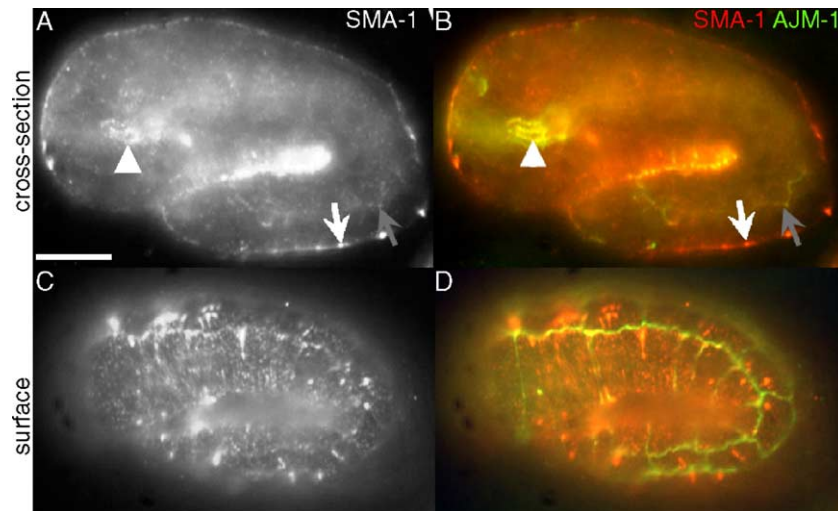


Fig. 2. SMA-1 organization at the apical membrane is dependent on functional SPC-1 α -spectrin. We co-stained the balanced strain DM3416 mnDp33/+IV; *spc-1(ra409)*X (A–D) with anti-SMA-1 antisera (A–D; red in B, D) and anti-AJM-1 antisera which recognizes a protein in AJ (green B, D) (Materials and methods). Late-stage embryos homozygous for *spc-1* that have lost the rescuing balancer were identified because they did not elongate past 2-fold but had mature pharynx structures (white arrowhead). A mid-sagittal (A, B) of a *Spc-1* embryo shows SMA-1 localizes to the apical membrane of the hypodermis (white arrow), gut (gray arrow), and pharynx (arrowhead), but the localization pattern is weaker and more punctate than in the wild type (compare Fig. 1H). A hypodermal surface view (C, D) confirms that SMA-1 is present on the surface, but less evenly dispersed than in the wild type (compare Fig. 1J). Scale bar in panel A, 10 μ m.

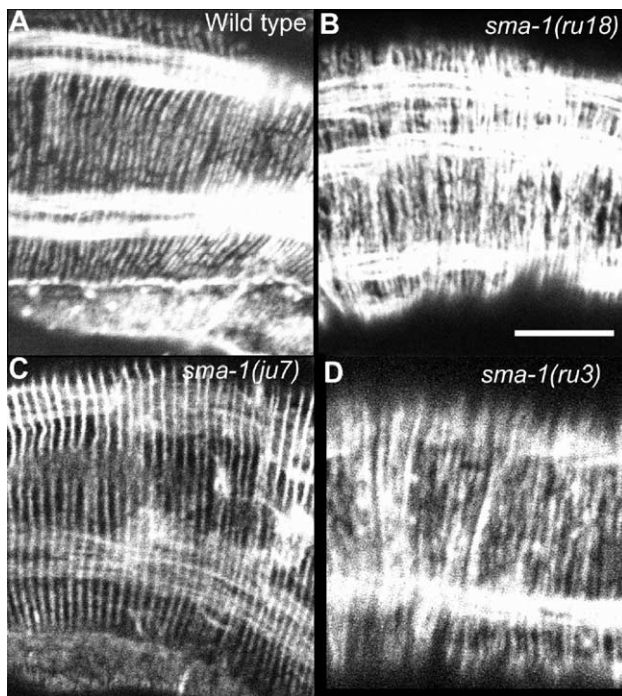


Fig. 3. Circumferential actin fibers in the hypodermis are disrupted in some but not all *sma-1* mutant embryos during morphogenesis. Wild type and *sma-1* embryos were stained with phalloidin. Muscles are visible as thick lines running anterior to posterior (left to right), perpendicular to the hypodermal cell actin fibers. (A) In wild type embryos in late-stage morphogenesis, the actin fibers in the dorsal hypodermis have a regular pattern of even intensity and spacing. This pattern is disrupted in Class I *sma-1(ru18)* null (B) and Class III *sma-1(ru3)* (D) embryos. Note the irregular spacing, variable intensity, and gaps due to out-of-focus actin fibers. In contrast, Class II *sma-1(ju7)* mutants (C) have well-organized actin fibers. Scale bar in panel B is 5 μ m.

and wild type embryos using a Z-stack analysis, which allowed us to examine the position of actin in distinct focal planes throughout an embryo. In wild type twofold embryos, CFs are in focus in only a few focal planes (Figs. 4A–C), and in-focus CFs are present from the dorsal hypodermis across the seam cells into the ventral hypodermis, extending well beyond the muscle quadrants (Fig. 4B arrows). We observed a similar pattern in later stage wild type embryos (data not shown). In contrast, in *sma-1(ru18)* embryos, only short stretches of CFs are in focus in a single focal plane and short stretches of in-focus CFs were present over a larger number of focal planes (Figs. 4G–L). CFs also never extended past the muscle quadrants in the dorsal/ventral hypodermis in *sma-1(ru18)* null embryos, but appeared to wrap around them (Figs. 4G–L). One explanation for these data is that a portion of the CFs have dissociated from the apical membrane in *sma-1(ru18)* mutants. In the thinnest portion of the hypodermis, above the muscle quadrants, the CFs are supported by the underlying muscle. In the thicker portions of the hypodermis, the CFs dissociate a greater distance from the apical membrane, which would explain their absence in regions that extend past the muscle quadrants. In both wild type and *sma-1(ru18)* embryos, the actin fibers remain intact at cell boundaries, unlike the phenotype observed in strains carrying defects in proteins of the AJ (Costa et al., 1998; Firestein and Rongo, 2001; Pettitt et al., 2003; Simske et al., 2003).

To determine if CFs are not associated with the apical membrane in *sma-1* embryos, we developed a method in which we could simultaneously examine actin organization and the apical membrane. AZ249 embryos, which carry an

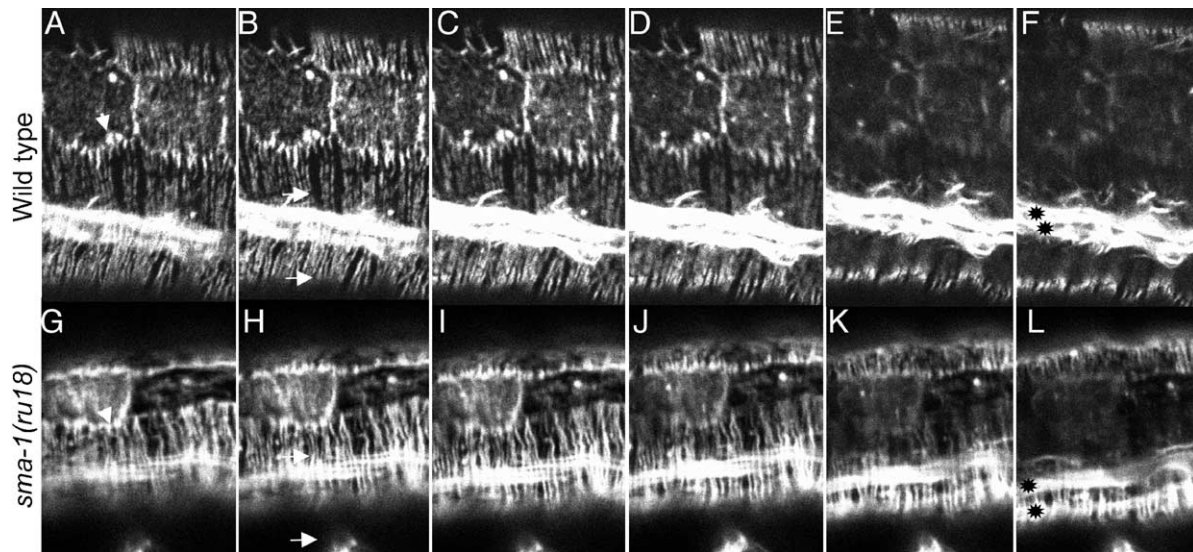


Fig. 4. CFs are mislocalized in *sma-1(ru18)* embryos. A confocal z-stack series of ~2-fold wild type (A–F) and *sma-1(ru18)* null (G–L) embryos stained with phalloidin, to visualize actin during cell elongation. Panels show ~2 cell widths of the hypodermis, starting at a focal plane slightly above the embryo and moving the same distance through each embryo. Anterior is to the left in each panel. In wild type embryos, CFs in the dorsal/ventral hypodermis are in focus on both sides of the muscle quadrants (white arrows, B), which stain as bright bands from left to right in each panel (black stars, F, L). These CFs are in focus in only three z-stack focal planes (A–C), indicating they are relatively planar. In the deeper cross-sections of wild type embryos, the focused CFs are present in a very narrow band some distance from the muscle fibers (E–F). In *sma-1(ru18)* null embryos, short stretches of CFs are in focus in five z-stacks (G–L), indicating a loss of planarity. In addition, the CFs appear to wrap around rather than extend past the muscle quadrants (black star, L), making the *sma-1(ru18)* embryo appear narrower than a wild type embryo at a similar stage of development (white arrows in panel H are the same distance apart as in panel B). These data are consistent with the interpretation that CFs have dissociated from the apical membrane and are now localized adjacent to the underlying muscle cells. Actin fibers at seam cell boundaries in *sma-1(ru18)* null embryos resemble the wild type (arrowheads in A, G), indicating these structures are intact. All confocal images used identical z-stack steps and aperture.

actin::GFP fusion construct that expresses protein in the hypodermis but not muscle cells during morphogenesis, were stained with the vital membrane dye FM4-64 to visualize the apical membrane (Materials and methods). These experiments show SMA-1 is required for the association of actin with the apical membrane during cell elongation. In *sma-1(+)* AZ249 embryos in late morphogenesis, actin::GFP forms a thin line of even intensity at the periphery of the embryo that co-localizes with the apical membrane of the hypodermis (Fig. 5). In *sma-1(RNAi)* AZ249 embryos at the same developmental stage, which appear twofold but have the mature pharyngeal structures of an older embryo, several defects are apparent. In embryos lacking SMA-1 activity, there are regions along the apical membrane of the hypodermis where actin::GFP is very sparse or completely absent (Fig. 5F, white arrowhead). In other regions of the hypodermis, GFP has a thicker pattern than the apical FM4-64 stain, indicating that while some actin is localized to the apical membrane, the remainder is in the cell interior. Some actin::GFP is also found associated with internal FM4-64 stain (Fig. 5F, white arrows). These data are consistent with the interpretation that CFs are generally disorganized and a subset are not associated with the apical membrane. These results show that, in the absence of SMA-1 spectrin, the association of actin with the apical membrane is disrupted in the elongating hypodermis.

The N-terminus of SMA-1 is sufficient to organize actin at the apical membrane

To better understand the function of SMA-1 and determine whether specific domains are required for distinct functions, we characterized 10 independent *sma-1* alleles to identify the changes in DNA sequence, measure hatchling length, and determine changes in CF organization using phalloidin (Materials and methods; Table 1). The *sma-1* alleles we characterized fell into 4 distinct classes (Table 1). The strong correlation between the position of the genetic lesion and the mutant phenotype demonstrates that SMA-1 protein has several independent functional domains.

Examination of the Class II alleles, with premature termination sequences early in the gene, indicates the N-terminus of SMA-1 is sufficient to organize actin during cell elongation. Class I *sma-1(ru18)* null mutants have a severe larval length phenotype and defects in the organization of CFs (Table 1, Fig. 3B). In contrast, Class II alleles, which have premature termination sequences early in the *sma-1* gene, prior to SR5, have a weak larval length phenotype and CF pattern identical to wild type (Table 1, Fig. 3 compare A, C). These data demonstrate the protein produced in Class II alleles is sufficient to organize actin at the apical membrane. One trivial explanation for these results is that the genetic lesion in Class II embryos is by-passed so that near full-length SMA-1 is produced. We discounted this possibility

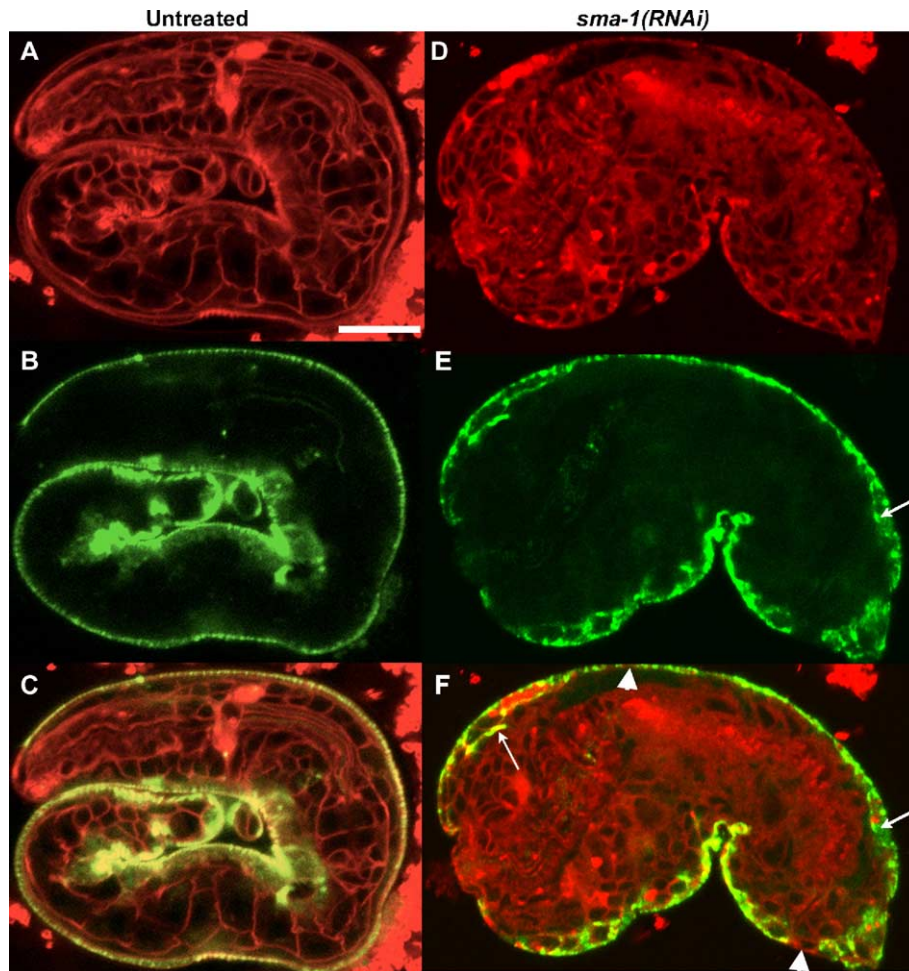


Fig. 5. The apical SBMS is disrupted in *sma-1(RNAi)* embryos. We examined FM4-64 membrane stain (A, D), Actin::GFP (B, E), and the merge (C, F) in the hypodermis of *sma-1(+)* (A–C) and *sma-1(RNAi)* (D–F) AZ249 embryos. AZ249 *sma-1(+)* and *sma-1(RNAi)* embryos, which are shorter due to elongation defects, are at a similar stage of development in these images. In a cross-section of *sma-1(+)* AZ249 embryos at the ~3-fold stage, the actin::GFP (B) is of even intensity and co-localizes in a narrow band with the apical plasma membrane of the hypodermis (A, C). In contrast, a cross-section of *sma-1(RNAi)* AZ249 embryos shows the actin::GFP pattern is severely disrupted, with a thicker and uneven pattern of localization (arrow, E). In the merge of the *sma-1(RNAi)*-treated embryo, there are clear instances of actin internal to the apical membrane (arrow) and regions with no actin at the apical membrane (arrowhead), a pattern not observed in untreated animals. These data are consistent with the interpretation that, in the absence of SMA-1 activity, actin fibers are generally disorganized and some are not associated with the apical membrane of hypodermal cells. In all photos, anterior is to the left, dorsal is at the top. Scale bar in panel A, 10 μ m.

because immunoblot and in situ staining experiments with antisera to either the SMA-1 SH3 or PH domains failed to recognize a protein product in Class II *sma-1(ju7)* embryos (Figs. 1M, O, data not shown). As the premature stop codon in *sma-1(ju7)* mutants is located in SR1 (Table 1, Fig. 1A), these data strongly support the hypothesis that the ability to organize actin at the apical membrane must reside in the amino-terminal CH domains of SMA-1.

To confirm that domains in the N-terminus of SMA-1 protein target SMA-1 to the apical membrane, we examined the localization of GFP produced from an integrated line carrying the SMA-1 SR4::GFP expression construct (Materials and methods). The SR4::GFP construct contains the sequence coding for the complete N-terminus of SMA-1, including a small portion of SR5, under the control of its native promoter, fused in frame to GFP (Fig. 1A). Stable

integrated lines carrying this construct, which are identical to the wild type with respect to larval length, express apically localized GFP in the hypodermis, gut, and pharynx (Figs. 6A, C). Although these results do not rule out the possibility that other domains in SMA-1 are important for apical localization of full-length protein, they demonstrate the amino-terminal region of SMA-1 containing the CH domains and SR1-4 is sufficient to properly localize SMA-1 to the apical membrane.

One possible explanation for the apical localization of the truncated SMA-1 protein is that it can interact with the apical network produced by full-length SMA-1 and SPC-1. To determine if an intact spectrin network is required to localize SR4::GFP to the apical membrane, we examined the SR4::GFP localization pattern in *sma-1(rul8)* null animals carrying the integrated SR4 construct. *sma-*



Fig. 6. The N-terminus of SMA-1 is sufficient to localize to the apical membrane. Wild type, *sma-1(ru18)* and *spc-1(ra409)* lines stably carrying the SR4::GFP construct (Fig. 1) were examined for GFP localization. SR4::GFP localizes to the apical membrane of the hypodermis, gut, and pharynx of both *sma-1*(+) (A, C) and *sma-1(ru18)* (B, C) and *spc-1(ra409)* embryos (C; Materials and methods). Because of technical considerations, it was not possible to both photograph and genotype *spc-1* animals carrying the SR4::GFP construct. Scale bar in panel A, 10 μ m.

l(ru18) mutant embryos have a defective apical cytoskeletal network due to the loss of SMA-1 activity. While the SR4::GFP construct does not rescue the *sma-1(ru18)* mutant length or actin organization phenotypes (data not shown), which may be due to inappropriate expression levels of the protein, SR4::GFP is properly localized to the apical membrane (Figs. 6B, C), indicating that an intact SBMS is not required for its localization.

Although SPC-1 α -spectrin is not required for the apical localization of full-length SMA-1, the organization of SMA-1 at the apical membrane is disrupted in *spc-1* mutants. To determine whether α -spectrin was required for apical localization of SR4::GFP, we examined the GFP localization pattern of this expression construct in *spc-1(ra409)* mutant embryos. We found that the α -spectrin mutants have apically localized SR4::GFP (Fig. 6C). These results indicate α -spectrin is not required for the apical localization of the truncated SMA-1. We conclude that the N-terminus of SMA-1 is sufficient for localization to the apical membrane of epithelial cells.

SMA-1 function is critical for cell shape change and morphogenesis

Although the N-terminus of SMA-1 can organize CFs, analysis of the four classes of *sma-1* mutants indicates that expression of full-length SMA-1 is required for embryonic elongation (Table 1). As described above, expression of a truncated SMA-1 protein in Class II alleles is sufficient to rescue disorganized CFs, but Class II embryos do not fully elongate. Class III alleles, which have premature stop codons late in the gene, also fail to fully elongate. Oddly, Class III alleles have a stronger phenotype than Class II alleles and have disorganized actin fibers (Fig. 3D), although both classes are predicted to express the N-terminal domain of the protein. We have not ascertained whether this conundrum is due to altered expression levels or altered function of truncated SMA-1. The phenotypes of Class III alleles are less severe than the Class I *sma-1(ru18)* null, indicating the expressed protein has some activity, and immunoblots show weak expression of a truncated product for some of these alleles (Table 1; E.C. and V.P. unpublished data). Since Class

I, II, and III alleles all fail to express the C-terminus of SMA-1, which contains the partial spectrin repeat essential for tetramerization with α -spectrin (Bennett and Baines, 2001) and the PH domain important for membrane association (Williams et al., 2004), one or both of these domains must be required for embryonic elongation.

Of the 10 mutants we examined, only the Class IV mutant *sma-1(ru7)* expresses normal amounts of full-length SMA-1 protein, as assayed by immunoblotting (data not shown). *sma-1(ru7)* contains a mutation that changes a conserved isoleucine to phenylalanine in the N-terminal CH domains (Table 1). *sma-1(ru7)* mutant embryos exhibit altered localization of SMA-1 spectrin (compare Figs. 7A, B to Figs. 1F–J). In the dorsal/ventral hypodermis, the signal at the apical membrane is stronger, thicker, and more punctate. Within the seam cells, we see a striking pattern of intensely stained discs, one per seam cell (Fig. 7B). Based on confocal analysis of animals double stained with DAPI, we determined these discs are not perinuclear (data not shown), but appear to be present on the cell surface.

The altered SMA-1 activity in *sma-1(ru7)* mutants changes the organization of the circumferential actin fibers. We stained *sma-1(ru7)* mutants with phalloidin to examine f-actin (Materials and methods). In wild type embryos, the seam cell hypodermis contains linear bundles of actin (Priess and Hirsh, 1986). In the seam cells of *sma-1(ru7)* embryos, actin is organized into rings, one per seam cell, which correspond to the altered SMA-1 protein (Fig. 7C). These data demonstrate that alterations in the SMA-1 protein that cause its mislocalization result in a commensurate mislocalization of actin fibers in the seam cell hypodermis. Since the mutation in *sma-1(ru7)* alters the CH domains in the N-terminus of SMA-1 spectrin, these results confirm that the CH domains of SMA-1 are essential for proper localization of actin at the apical membrane.

Because actin organization is altered in the seam cells of *sma-1(ru7)* mutants, we predicted that the animals would have cell shape defects. In wild type hatchlings, the rectangular seam cells are found in one row on each side of the embryo (Priess and Hirsh, 1986). In *sma-1(ru7)* hatchlings, the seam cells remain organized in rows (data not shown) but their shape is altered. In scanning electron

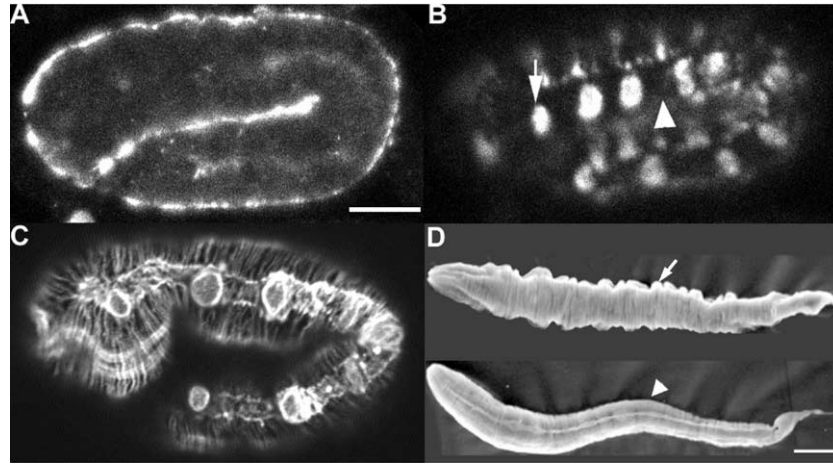


Fig. 7. In *sma-1(ru7)* mutant embryos, SMA-1 is mislocalized, leading to changes in actin organization and cell shape defects. In *sma-1(ru7)* mutant embryos stained with anti-SMA-1 PH antisera (A, B), SMA-1 localizes to the apical cell surface of hypodermal cells (A), but the pattern is thicker and more punctate than in wild type animals (compare Figs. 1F, H). In the lateral cells (B), SMA-1 forms discs (arrows), and the staining at the cell boundaries is no longer continuous (arrowheads), as in the wild type (compare Fig. 1J). In *sma-1(ru7)* mutants stained with phalloidin (C), the pattern of actin is clearly disrupted in the seam cells, with more subtle changes in the dorsal ventral hypodermis. A ring or disc of actin is observed in each of the lateral cells of the embryo. A scanning electron micrograph of a *sma-1(ru7)* mutant larvae just after hatching (D) show dramatic alterations in the shape of the lateral hypodermis (arrow) while the dorsal and ventral hypodermis (arrowhead) remains relatively unaffected. In panels A–C, anterior is to the left; dorsal is at the top. In panel D, the upper animal is a dorsal view; the lower animal is a lateral view. Scale bar in panels A (applies to panels B, C) and D, 10 μ m.

micrographs, we found the seam cells of *sma-1(ru7)* hatchlings formed bulges on the sides of the embryo (Fig. 7D). We believe this altered cell shape occurs because mislocalized SMA-1 causes actin fibers to form a ring. When this ring of actin contracts, the portion of the apical cell membrane encircled by actin protrudes from the embryo. Because *sma-1(ru7)* embryos produce full-length SMA-1, this altered cell shape is then stabilized by the SBMS. That mislocalized SMA-1 protein results in cellular deformations demonstrates that SMA-1 protein plays a critical role in stabilizing cell shape changes required for morphogenesis in the *C. elegans* embryo.

Discussion

SMA-1 stabilizes the association between actin and the apical membrane

SMA-1 spectrin, an ortholog of β_H -spectrin, is essential for cell shape change of polarized epithelial cells during *C. elegans* development. SMA-1 localizes to the apical membrane of cells of the pharynx, gut, hypodermis, and excretory canal (Fig. 1) during the stages of development when these cells are rapidly elongating. In the hypodermis, just prior to cell elongation, SMA-1 localizes to the apical membrane simultaneously with the reorganization of actin into apically localized bundles that circumscribe the embryo (Fig. 1, Priess and Hirsh, 1986). SMA-1 does not have a primary role in directing the reorganization of actin, as it occurs in *sma-1(ru18)* null mutants, but SMA-1 is essential in maintaining the association of actin bundles with the apical membrane during cell elongation. In the absence of

SMA-1 activity, the actin bundles are not stably associated with the apical membrane (Figs. 3–5). Previous work has shown the actin fibers associated with the apical membrane of the hypodermal cells are essential for cell elongation during *C. elegans* morphogenesis (Priess and Hirsh, 1986). We believe one major reason hypodermal cells do not elongate properly in *sma-1(ru18)* null mutants is that the force generated from contraction of these actin bundles is unable to act on the apical membrane skeleton because the two structures are no longer stably attached to each other.

The interaction between SMA-1, actin, and the apical membrane occurs due to interactions in the N-terminus of SMA-1 protein and is independent of α -spectrin. In the Class III *sma-1(ju7)* mutant, which contains a premature termination sequence in SR1 that eliminates the α -spectrin binding domain (Begg et al., 2000), the actin fibers are identical to wild type, indicating a normal association between actin and the apical membrane (Fig. 3). The SR4::GFP expression construct, which contains the 2 CH domains and SR1–4 at the N-terminus of SMA-1 (Fig. 1A), localizes GFP to the apical membrane in a pattern identical to that of full-length SMA-1 protein. This normal localization pattern is maintained in both *spc-1(ra409)* and *sma-1(ru18)* mutant embryos (Fig. 6), demonstrating the interaction does not require α -spectrin or an intact spectrin network. These results are consistent with a model for CH domain function, based on other actin-binding proteins, that proposes the CH domains interact with actin and other proteins (Banuelos et al., 1998; Bennett and Baines, 2001). Recent work in *Drosophila* has demonstrated the C-terminus of β_H -spectrin is sufficient to target the protein to all cell membranes (Williams et al., 2004). Our results show the N-terminal CH domains of SMA-1 are sufficient

to localize SMA-1 to the apical membrane and to maintain organization of actin during cell elongation.

The proteins essential for the interaction between SMA-1 and the apical membrane have not yet been identified. Although SPC-1/ α -spectrin is not required for its apical localization, the organization of SMA-1 is altered in *spc-1* mutants (Fig. 2). Other candidates include transmembrane proteins such as CRB-1, the *C. elegans* *crumbs* ortholog, which co-immunoprecipitates with the ortholog of SMA-1 in *Drosophila* (Medina et al., 2002), ERM-1, which shows a genetic interaction with *sma-1* (Gobel et al., 2004) or scaffolding proteins such as PAR-3, PAR-6, or PKC-3 (reviewed in Knust and Bossinger, 2002). However, the relationship between SMA-1 and these proteins, if any, remains to be elucidated. Other as-yet-unidentified proteins may also function in *C. elegans* to localize SMA-1 to the apical membrane.

SMA-1 is a component of an apical spectrin-based membrane skeleton

The SMA-1 SBMS is also essential to make changes in actin organization and cell shape permanent. The evidence for this activity comes from examination of the four classes of *sma-1* mutants. Unlike the Class I *sma-1(ru18)* null mutant, Class II *sma-1(ju7)* mutant embryos have organized CFs (Fig. 3) ensuring force from the actin contractile apparatus can act on the apical membrane. However, we believe Class II mutant embryos fail to elongate properly because, in the absence of full-length SMA-1, this contractile force does not have an intact SBMS upon which to act, preventing permanent changes in cell shape. Class III mutants, which also fail to fully elongate, contain premature termination sequences prior to the region thought required for tetramerization (Bennett and Baines, 2001) suggesting a SMA-1 spectrin network is crucial for cell elongation. Only in the Class IV *sma-1(ru7)* mutant embryos, is full-length network-competent SMA-1 expressed. In *sma-1(ru7)* embryos, mislocalized SMA-1 results in mislocalized actin, and these changes in the SMA-1 SBMS cause permanent perturbations in cell shape (Fig. 7). These data show that the SMA-1 SBMS acts to stabilize changes in actin organization, resulting in permanent cell shape change during morphogenesis.

SMA-1 spectrin's role in stabilizing changes in the apical membrane skeleton requires interactions with α -spectrin. The evidence that SMA-1/ β_H -spectrins interact with α -spectrin to form a heterotetramer is well established from earlier genetic, structural, and protein localization studies in *Drosophila* and *C. elegans* (Dubreuil et al., 1997; McKeown et al., 1998; Norman and Moerman, 2002; Thomas and Williams, 1999; Zarnescu and Thomas, 1999) and is further supported by the results reported here. The apical localization pattern we observe for SMA-1 spectrin is similar to that observed for a subset of SPC-1 (Fig. 1, Norman and Moerman, 2002), the only *C. elegans* α -

spectrin, consistent with a proposed interaction between the two proteins. Although the localization of SMA-1 at the apical membrane does not absolutely require SPC-1 α -spectrin, SMA-1 organization is altered in *spc-1* mutants, consistent with an interaction between the two proteins. These findings and the similar phenotypes of *spc-1* and *sma-1* mutant embryos with respect to cell elongation (McKeown et al., 1998; Norman and Moerman, 2002) indicate SMA-1 and α -spectrin form an apically localized spectrin network that fails to fully function if either protein is absent.

SMA-1 is not essential for cell adhesion

In *Drosophila*, the SMA-1 ortholog β_H -spectrin localizes to and plays an essential role in adhesion junctions (Thomas et al., 1998; Zarnescu and Thomas, 1999). The role of SMA-1 spectrin in adhesion junctions is still unclear. While the SMA-1 spectrin localization pattern is clearly distinct from the AJ protein AJM-1, localizing predominantly to the apical membrane of the epithelial cells rather than to AJs, we cannot exclude the possibility that there is overlap in expression in some tissues (Fig. 1K). *sma-1* mutants exhibit no visible cell adhesion defects (McKeown et al., 1998) while loss of function mutations in known adhesion junction proteins results in embryonic arrest (Bossinger et al., 2001; Costa et al., 1998; Firestein and Rongo, 2001; Hoier et al., 2000; Koppen et al., 2001; Pettitt et al., 2003; Raich et al., 1999). The nature of the disruption of actin fibers in *sma-1* null mutants is also distinct from that observed in strains with defects in cell adhesion junction proteins. In *hmp-1* or *dlg-1(RNAi)* embryos, actin fibers dissociate from the lateral cell boundary during morphogenesis (Costa et al., 1998; Firestein and Rongo, 2001). In *sma-1* null mutants, the actin fibers remain associated with the lateral cell boundaries, but pull away from the apical membrane (Figs. 3–5). These results indicate that SMA-1 spectrins' primary role is not the attachment of actin to the adhesion junction but between actin and the apical cell membrane. It is not clear why *Drosophila* β_H -spectrin has a primary role in cell adhesion and the *C. elegans* ortholog SMA-1 does not. There may be a fundamental difference between the epithelial cells of *C. elegans* and *Drosophila*, between *sma-1* and *karst* β_H -spectrin function in these systems, or in the adhesion junction structure. Further experimentation will be necessary to elucidate the precise role SMA-1 plays in the adhesion junctions.

Model for SMA-1 function

We propose the following model for SMA-1 function in cell elongation during *C. elegans* morphogenesis. At the beginning of cell elongation, SMA-1 spectrin interacts with α -spectrin to form a tetrameric network that cross-links actin to the apical membrane (Fig. 8A). The binding of SMA-1 to the apical membrane may require the cooperative activities

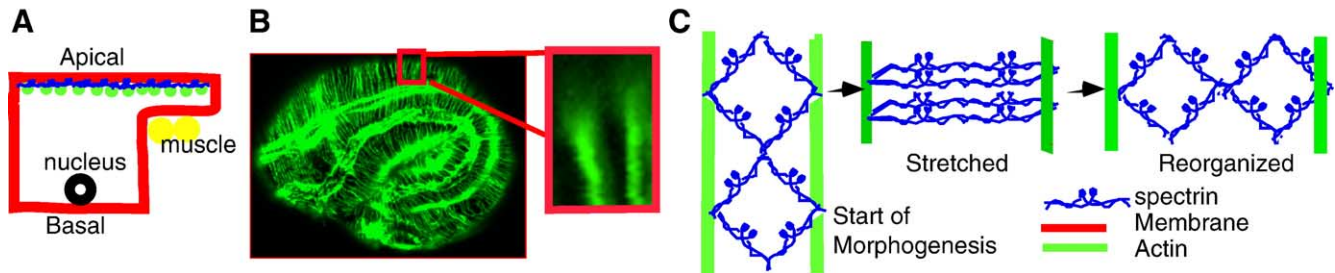


Fig. 8. Model for SMA-1 function. SMA-1 has several independent roles in cell shape change and morphogenesis. (A) A schematic cross-section of a hypodermal cell with associations SMA-1 (blue) and actin (green) is shown. Cell boundaries are in red. Note that the region above the muscles (yellow) is thin. (B) A wild type embryo stained with phalloidin to visualize actin. Red box indicates a region that is amplified in the inset, which shows two parallel fibers localized to the apical membrane. (C) Model for SMA-1 function showing a schematic of the actin fibers as in panel B, inset. As actin reorganizes into parallel bundles during morphogenesis, SMA-1 is required to maintain the localization of actin at the apical membrane. As the embryo elongates, the SMA-1 spectrin network stretches to accommodate changes in cell shape. To stabilize these changes, the SMA-1 spectrin network dynamically reorganizes, returning to its relaxed state.

of the N-terminus and the C-terminus (Williams et al., 2004). As actin-myosin fibers constrict, SMA-1 spectrin maintains the attachment of actin to the apical membrane and provides the membrane with a structural matrix upon which the force of contraction acts. As the hypodermal cell begins to elongate, the actin bundles (Fig. 8B) move apart from each other (Costa et al., 1997), causing the associated SMA-1 spectrin network to shift from a relaxed to a stretched state (Fig. 8C). Because the β_H -spectrin tetramer is not sufficiently long to accommodate the maximum distance between the actin fibers (Costa et al., 1997; Dubreuil et al., 1990), we believe one end of the SMA-1 spectrin tetramer dissociates from the apical membrane, the tetramer returns to a relaxed state, and the tetramer re-associates with the apical membrane in a new location (Fig. 8C). Maintaining some association between the spectrin tetramer and the apical membrane during the transition between the stretched and newly relaxed states may require α -spectrin, other domains of SMA-1 including the C-terminus, or cooperative dissociation and re-association between the two ends of the tetramer. By creating a newly relaxed SMA-1 spectrin skeleton (Fig. 8C), changes in cell shape are stabilized.

Our model for SMA-1 function predicts the association between SMA-1 spectrin and the apical membrane is responsive to changes in the actin bundles or cell shape. Evidence from erythrocytes suggests the spectrin tetrameric network is dynamic and can reorganize under distortional conditions (Liu et al., 1982). Unlike the erythrocyte, the SMA-1 SBMS must reorganize in a very short period of time, by responding to signals within the elongating hypodermal cell. This necessitates that the association of SMA-1 spectrin with the apical membrane is regulated and dynamic.

We have not yet identified the domains of SMA-1 protein that respond to signaling cues, although analysis of *sma-1* mutant alleles offers tantalizing clues. Class III mutants have more severe phenotypes than Class II mutants (Table 1), an unexpected result because both classes of mutants express the N-terminus found sufficient to stabilize actin at the apical membrane. One explanation for this puzzle is that

Class III alleles express a domain of SMA-1 that normally regulates the association between SMA-1, actin, and the apical membrane. Under normal conditions, actin fibers that dissociate from SMA-1 at the apical membrane during network remodeling are held in place through other interactions in the SBMS. In the absence of this network, as is the case for Class III alleles, actin fibers would respond to a signal mediated through SMA-1 domains expressed in Class III alleles, and dissociate from the apical membrane, preventing embryonic elongation. Consistent with this hypothesis is the data showing that actin fibers are disrupted in *spe-1(lf) sma-1(+)* embryos (Norman and Moerman, 2002), implying full-length SMA-1 is not sufficient to maintain the organization of actin on its own. Because other interpretations of these data are possible, additional experimentation will be required to test this aspect of the model for SMA-1 function.

The experimental results presented here demonstrate that SMA-1 spectrin has several essential functions for cell elongation. SMA-1 spectrin is required for the localization of actin to the apical membrane in elongating epithelial cells. The N-terminus of SMA-1 spectrin is sufficient for the interactions between SMA-1, CFs, and apical membrane. SMA-1, interacting with α -spectrin, also forms an SBMS that stabilizes changes in the shape of the apical membrane, allowing progressive changes in cell shape. These coordinated functions of SMA-1 protein are essential for cell shape change and morphogenesis in the *C. elegans* embryo.

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